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## **Synthetic analogues of huwentoxin-IV spider peptide with altered human $Na_v1.7/Na_v1.6$ selectivity ratios**

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Huwentoxin-IV (HwTx-IV), a peptide discovered in the venom of the Chinese bird spider *Cyriopagopus schmidtii*, has been reported to be a potent antinociceptive compound due to its action on the genetically validated  $Na_v1.7$  pain target. Using this peptide for antinociceptive applications *in vivo* suffers from one major drawback, namely its negative impact on the neuromuscular system. Although studied only recently, this effect appears to be due to an interaction between the peptide and the  $Na_v1.6$  channel subtype located at the presynaptic level. The aim of this work was to investigate how HwTx-IV could be modified in order to alter the original human (h)  $Na_v1.7/Na_v1.6$  selectivity ratio of 23. Nineteen HwTx-IV analogues were chemically synthesized and tested for their blocking effects on the  $Na^+$  currents flowing through these two channel subtypes stably expressed in cell lines. Dose-response curves for these analogues were generated, thanks to the use of an automated patch-clamp system. Several key amino acid positions were targeted owing to the information provided by earlier structure-activity relationship (SAR) studies. Among the analogues tested, the potency of HwTx-IV E<sup>4</sup>K was significantly improved for h $Na_v1.6$ , leading to a decreased h $Na_v1.7/hNa_v1.6$  selectivity ratio (close to 1). Similar decreased selectivity ratios, but with increased potency for both subtypes, were observed for HwTx-IV analogues that combine a substitution at position 4 with a modification of amino acid 1 or 26 (HwTx-IV E<sup>1</sup>G/E<sup>4</sup>G and HwTx-IV E<sup>4</sup>K/R<sup>26</sup>Q). In contrast, increased selectivity ratios (> 46) were obtained if the E<sup>4</sup>K mutation was combined to an additional double substitution (R<sup>26</sup>A/Y<sup>33</sup>W) or simply by further substituting the C-terminal amidation of the peptide by a carboxylated motif, linked to a marked loss of potency on h $Na_v1.6$  in this latter case. These results demonstrate that it is possible to significantly modulate the selectivity ratio for these two channel subtypes in order to improve the potency of a given analogue for h $Na_v1.6$  and/or h $Na_v1.7$  subtypes. In addition, selective analogues for h $Na_v1.7$ , possessing better safety profiles, were produced to limit neuromuscular impairments.

**Keywords:** huwentoxin-IV, selectivity, sodium channel.